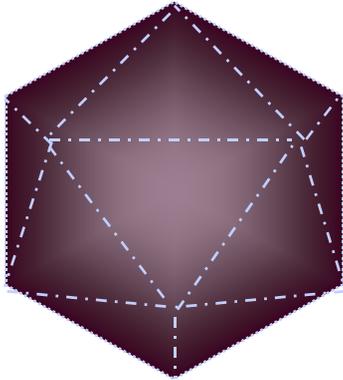


- 1. Wild-type AAV**
- 2. Recombinant AAV Vectors**
- 3. rAAV Vaccines**
- 4. Emerging Concepts**

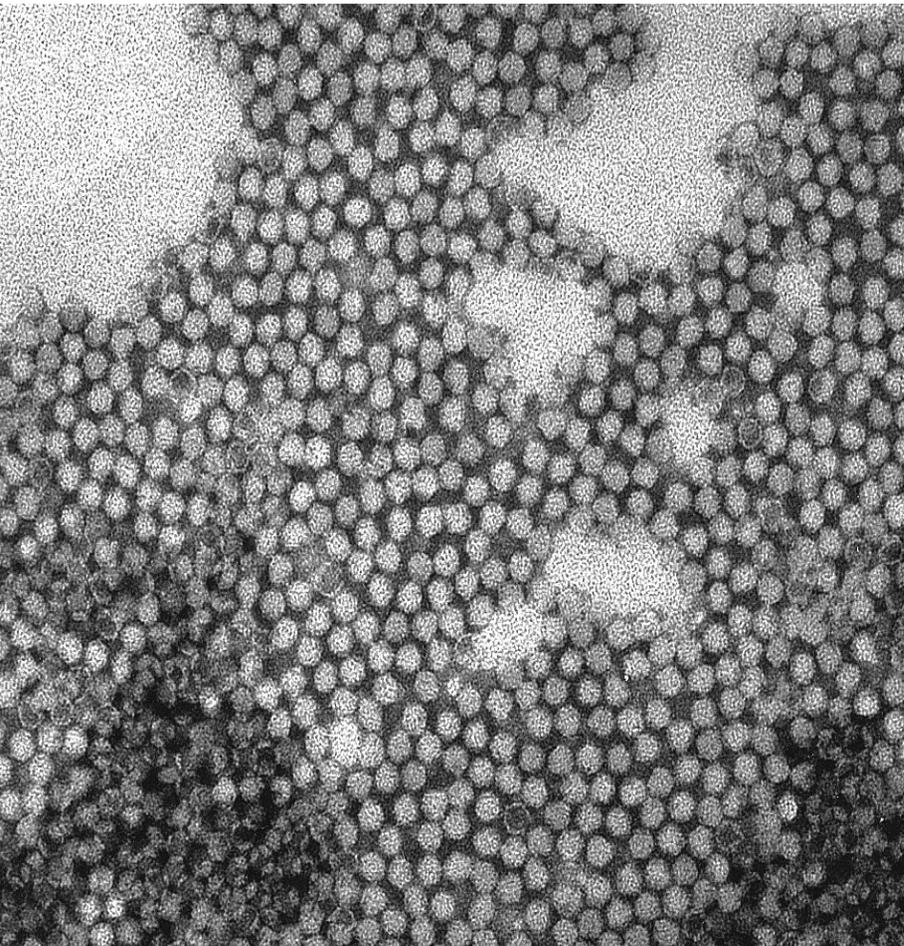
1. Wild-Type AAV

Wild-Type AAV

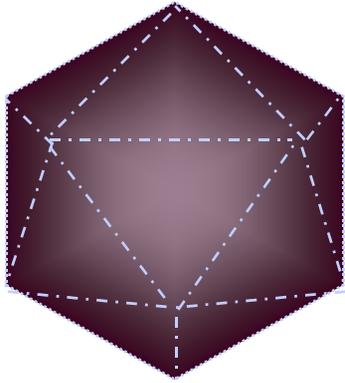
- **Replication incompetent**
- **Avirulent, non-pathogenic**
- **Requires helper virus to reproduce**
- **Naturally acquired infections early in life (with helper virus)**
- ***In vivo* biology is an emerging story**



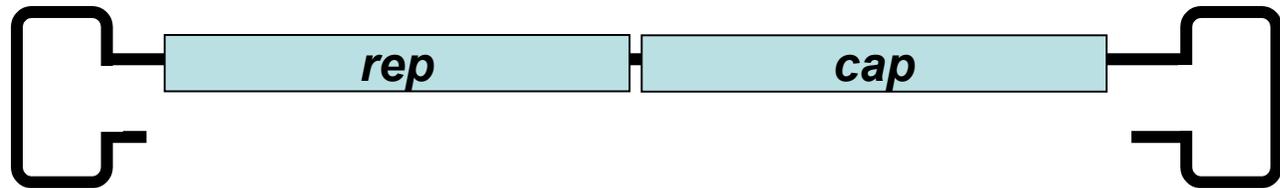
The AAV Particle



- **Parvovirus**
- **25 nm virion**
- **Non-enveloped**
- **Icosahedral capsid**
- **VP1, VP2, VP3**



The AAV Genome

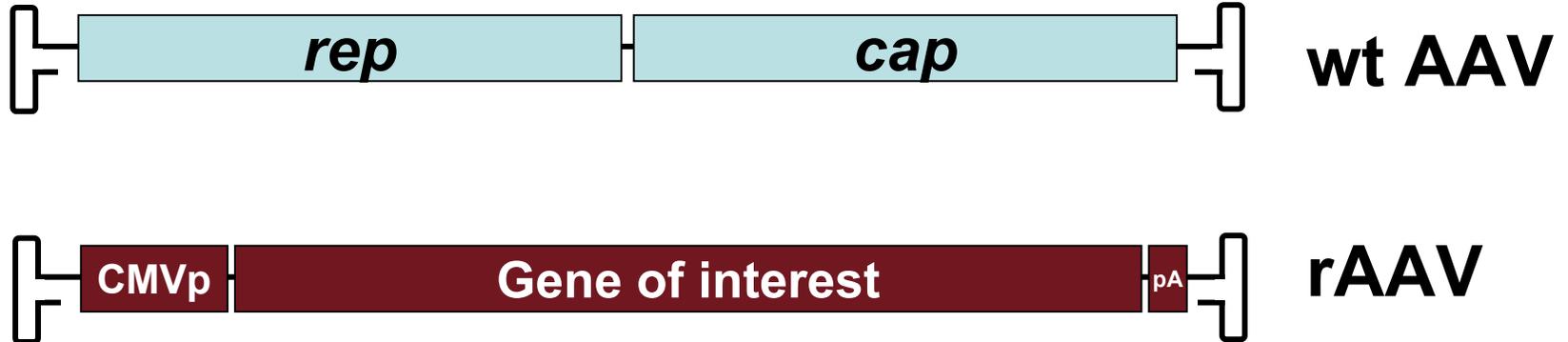


4680 nt

2. Recombinant Vectors

rAAV Vectors Are Simple

- Based on wild-type AAV
- Devoid of any AAV genes



Contains < 400 nt of AAV DNA

rAAV Vectors Are Highly Efficient Transduction Agents

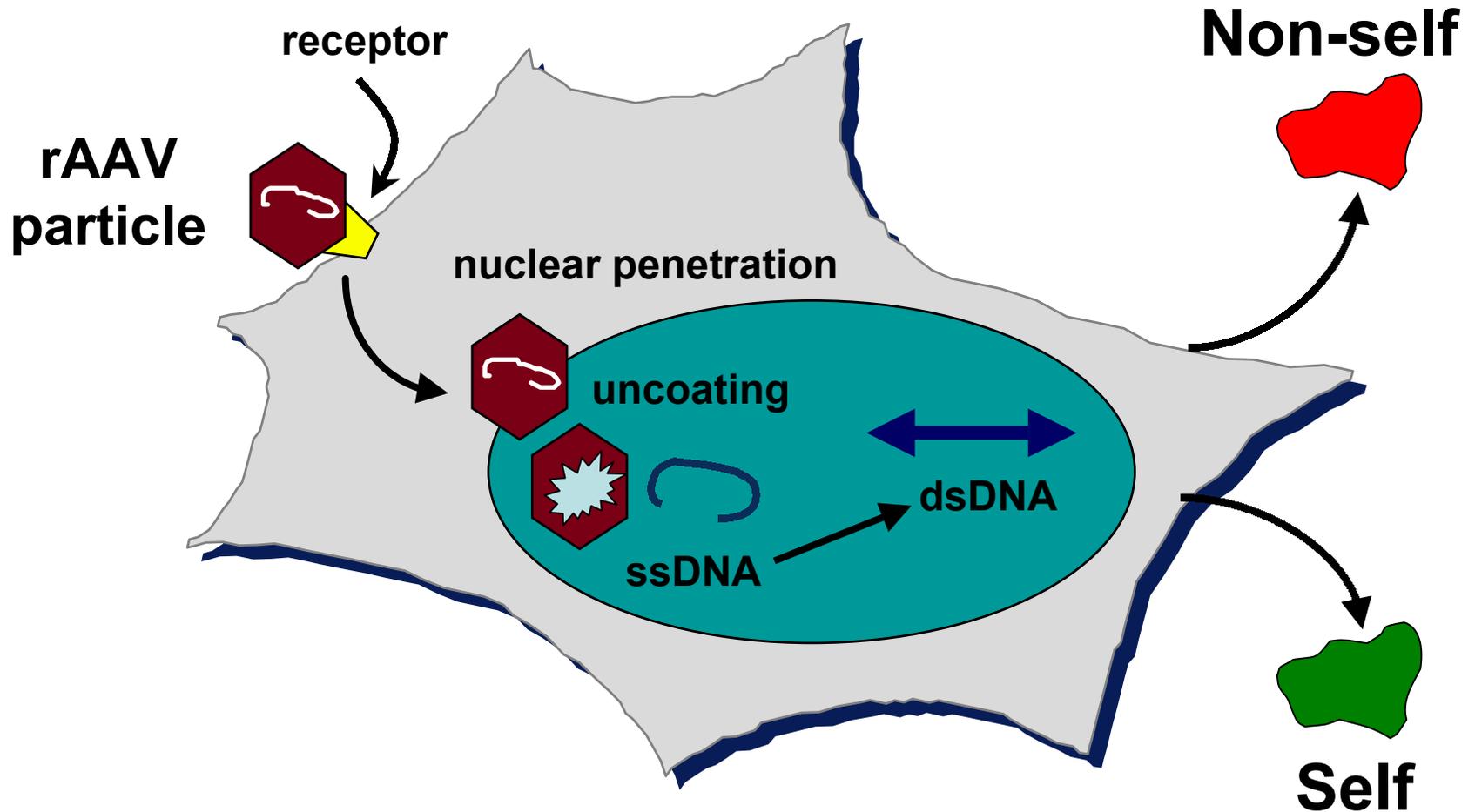


Ambient



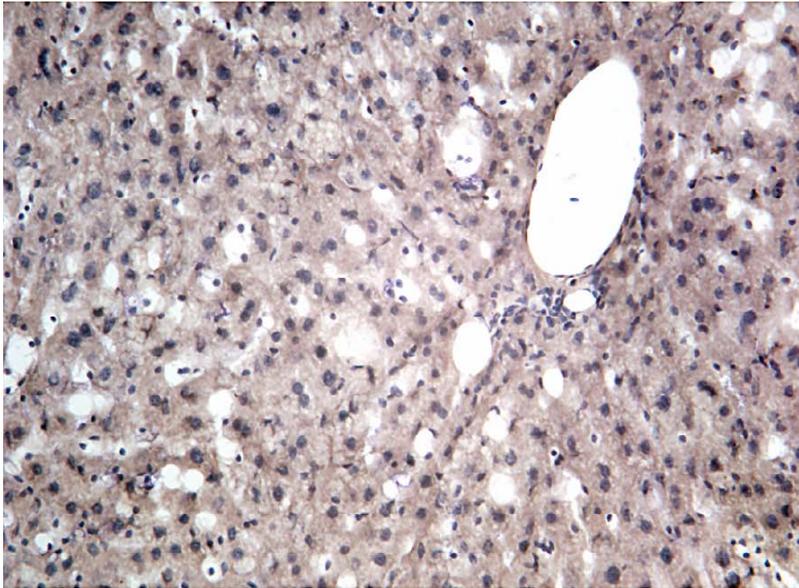
UV

rAAV is a DNA Delivery Vehicle

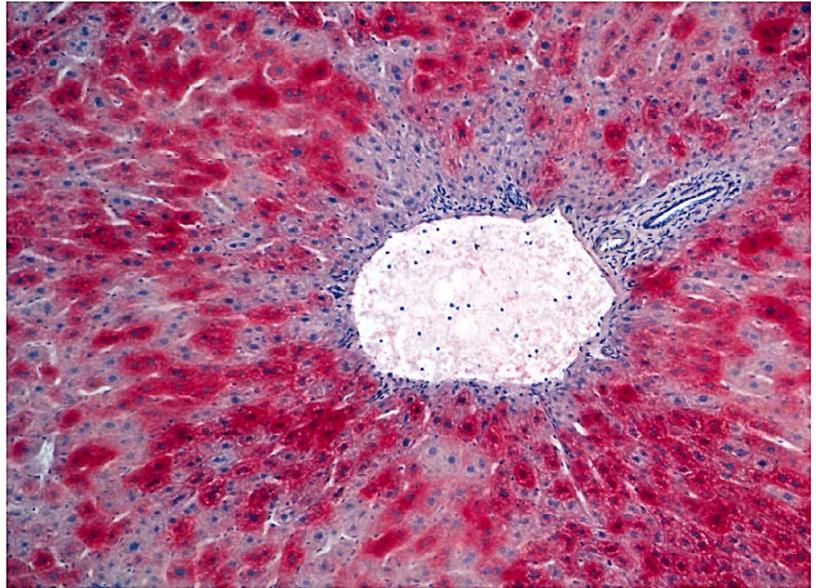


Delivery of a Self Gene

rAAV2/ β -glucuronidase in mice (liver)



Mock

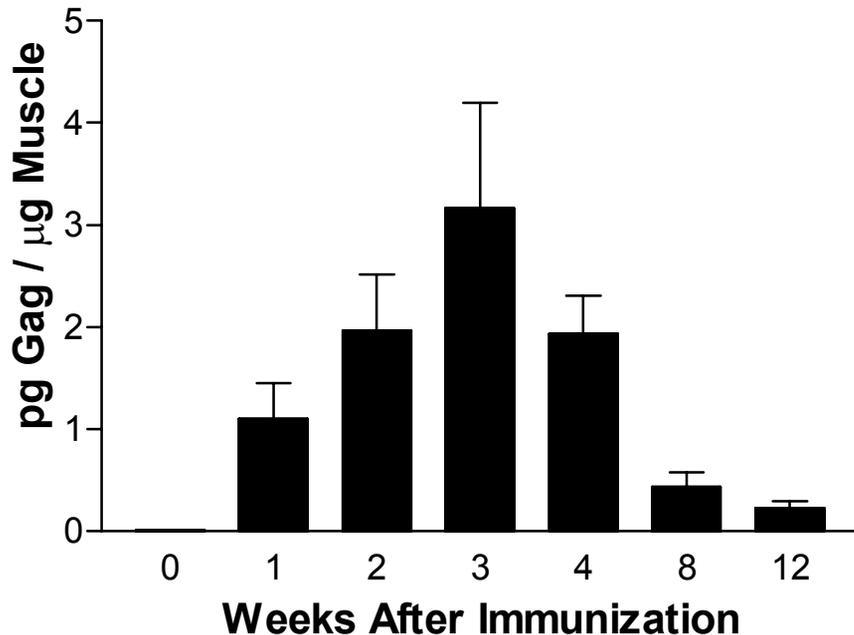


Treated

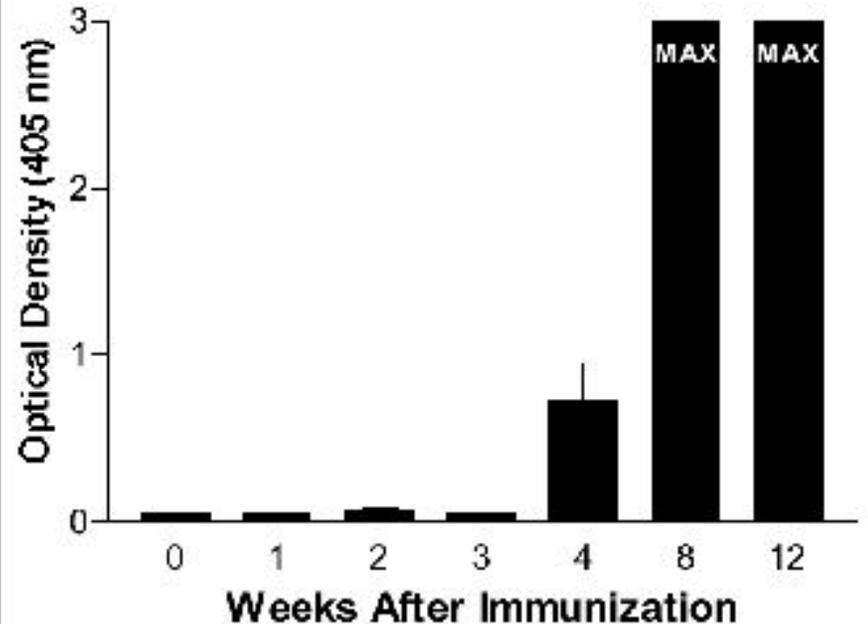
Delivery of a Non-self Gene

rAAV2/HIV-1gag in mice (muscle)

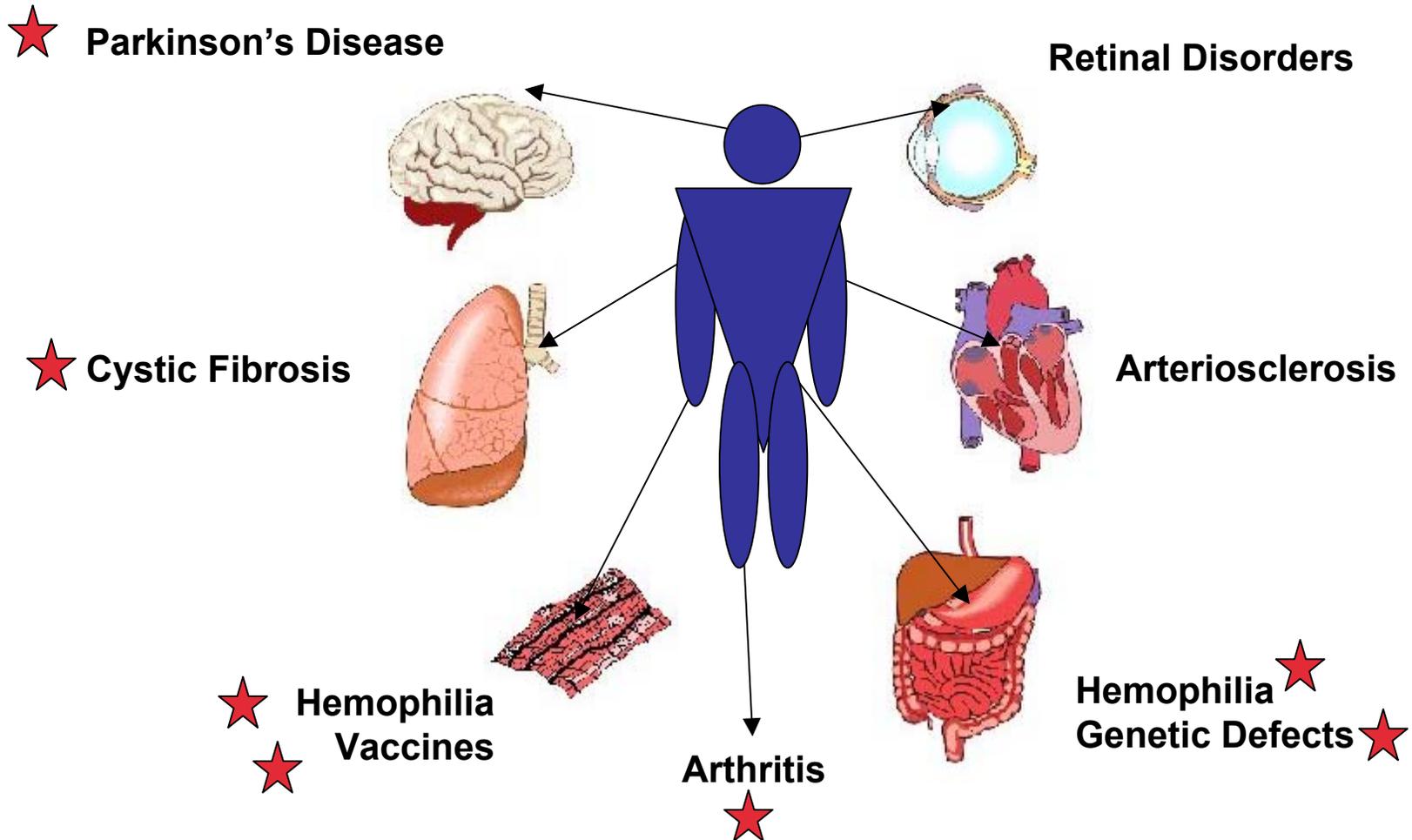
Gag Protein in Muscle



Gag Serum Antibodies



Clinical Application of rAAV Vectors

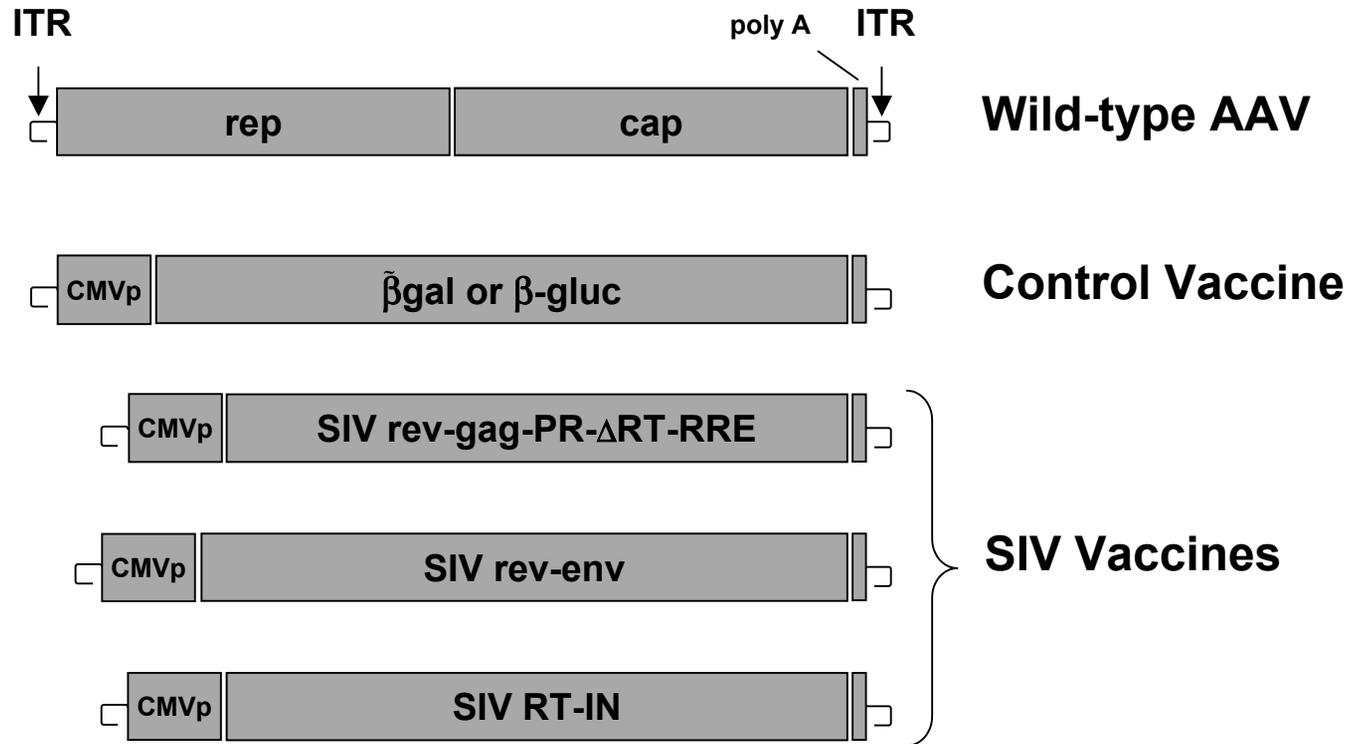


3. rAAV Vaccines

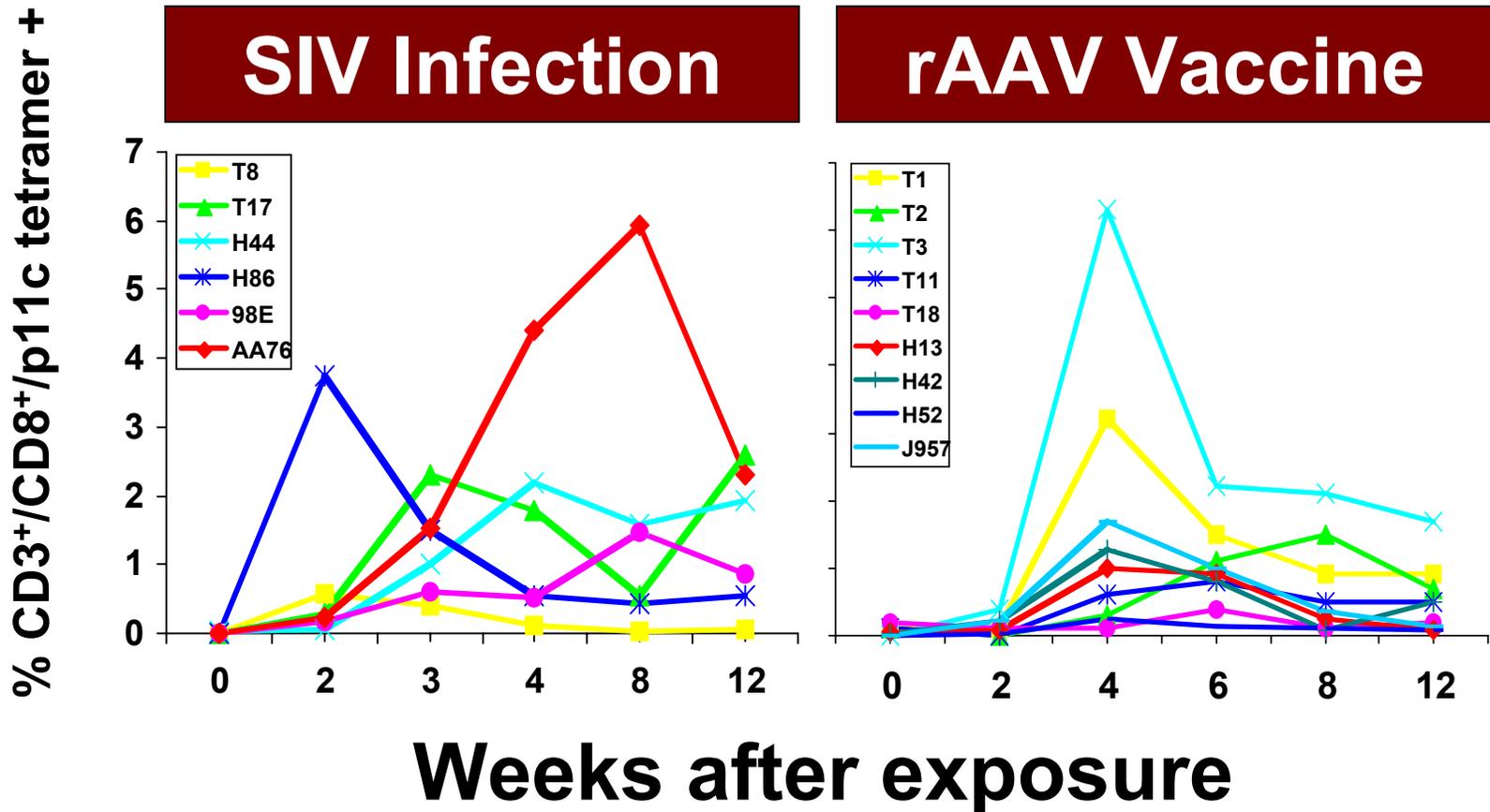
rAAV Vectors as Vaccines

- **Efficient transduction of target cells**
- **Elicits desirable immune responses**
- **Non-reactogenic, safe**
- **Scalable, affordable production**
- **SIV model system for HIV**

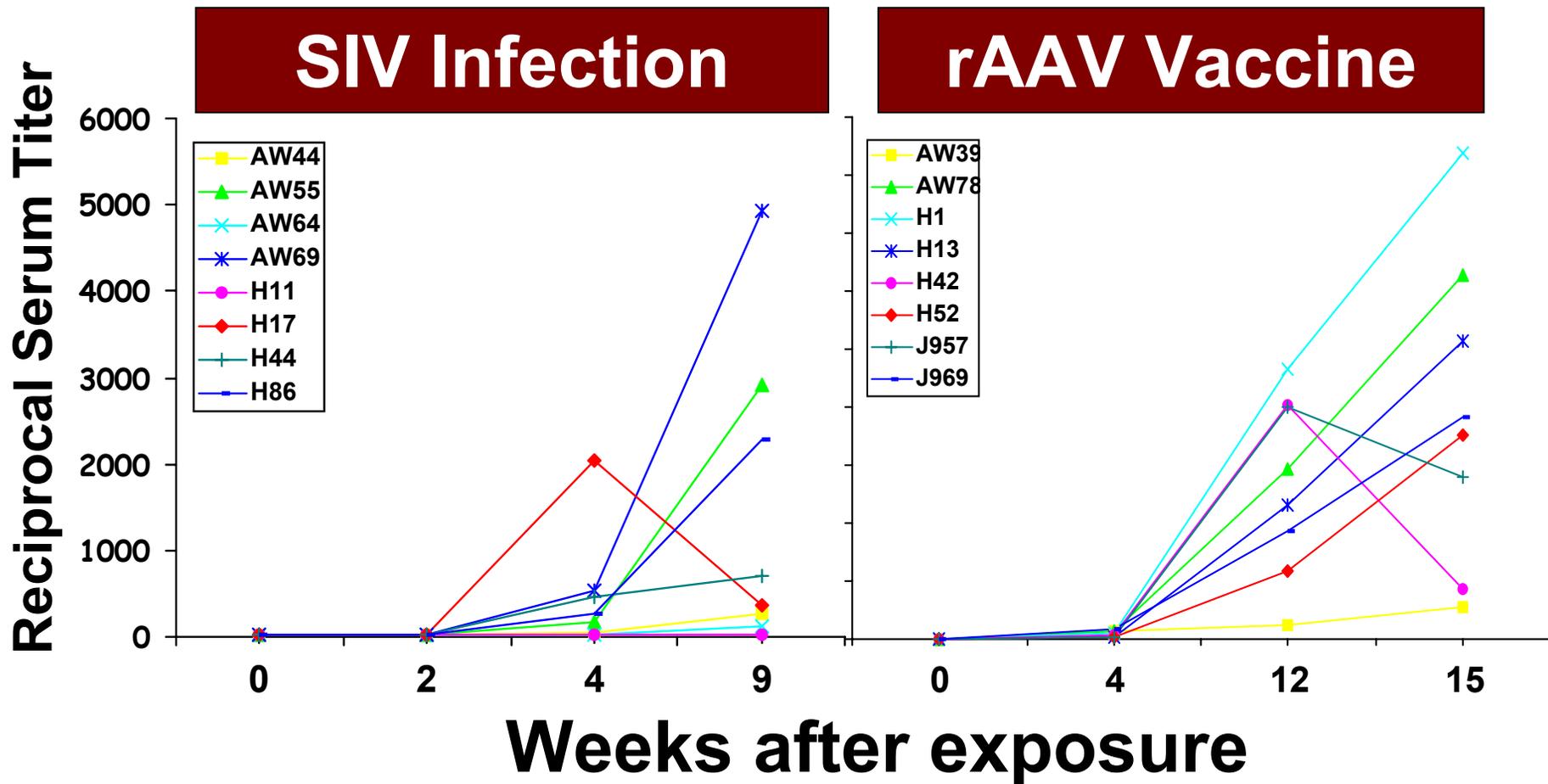
rAAV2/SIV Vaccine Constructs



rAAV/SIV Elicits Antigen-specific T cells in Macaques



rAAV/SIV Elicits Antigen-specific Antibodies in Macaques



rAAV/SIV Vaccine Restricts Replication of SIV in Macaques

- **3 separate studies, iv challenge**
- **SIVsm/E600 pathogenic challenge**
- **Low dose challenge - protection**
- **High dose challenge (low viral loads)**

HIV-1 Vaccine Construct



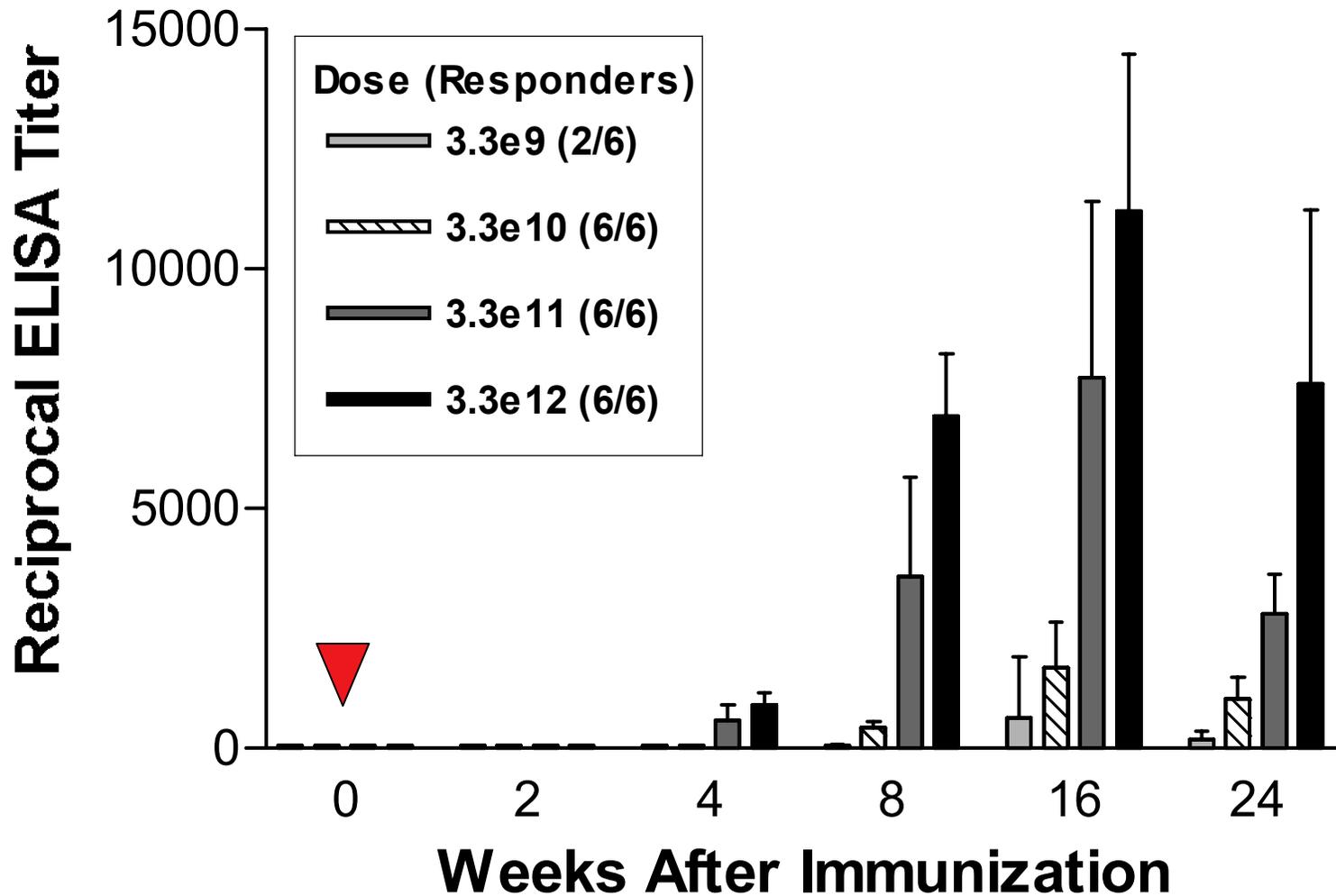
- **Co-developed by CCRI, TGC, IAVI**
- **Clade C (Carolyn Williamson)**
- **AAV serotype 2 capsid**

Preclinical Evaluation

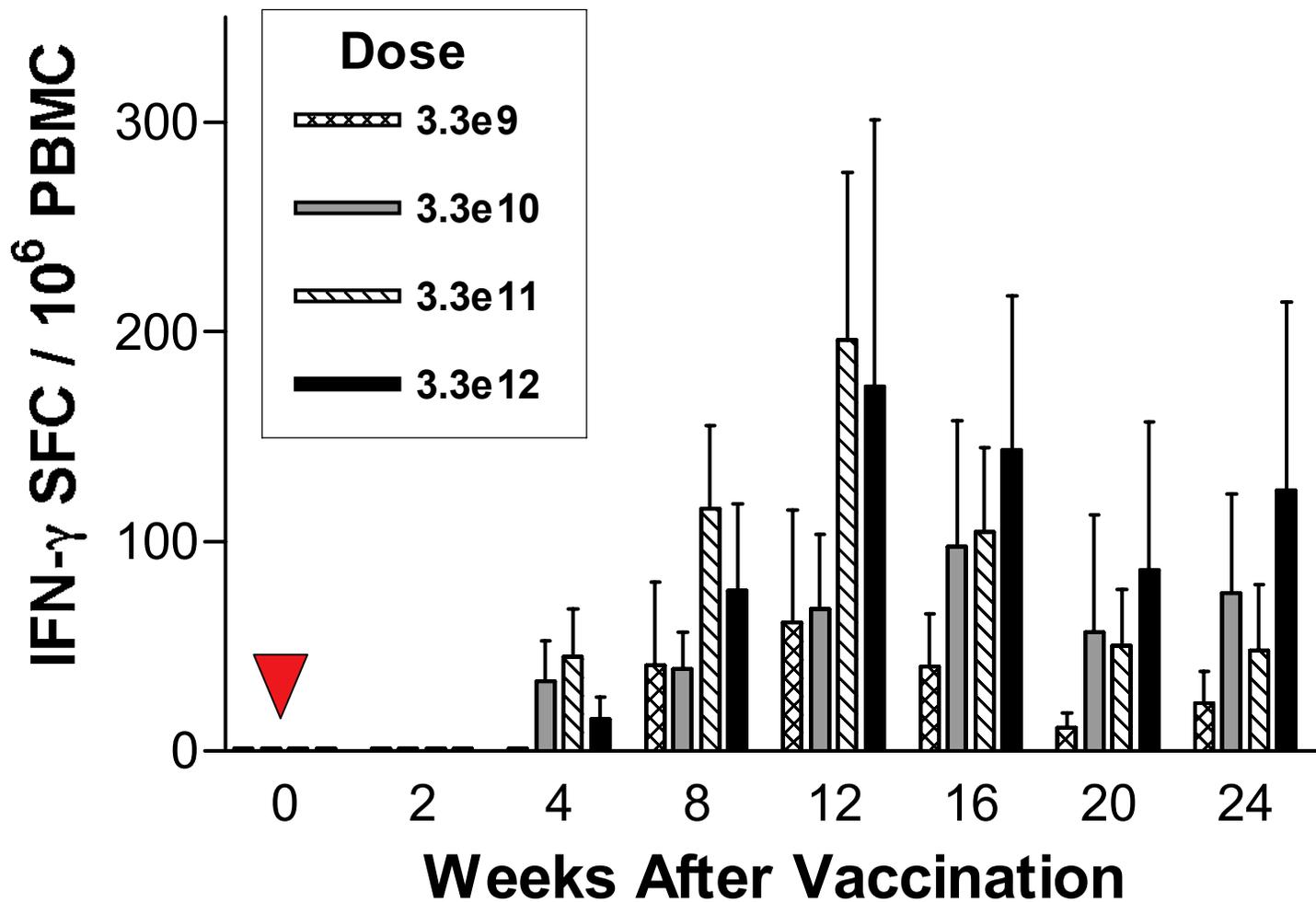
Immunogenicity

- **Dose finding study (macaques)**
- **Single im inoculation**
- **Antibodies against Gag**
- **ELIspot vs single peptide pool**

Antibodies Against Gag in Monkeys



ELIspot Responses in Monkeys



Preclinical Evaluation

Safety and Biodistribution

- **Rabbits and macaques**
- **Vaccine was well tolerated**
- **Vector distributed as expected**
- **Integration not detected**

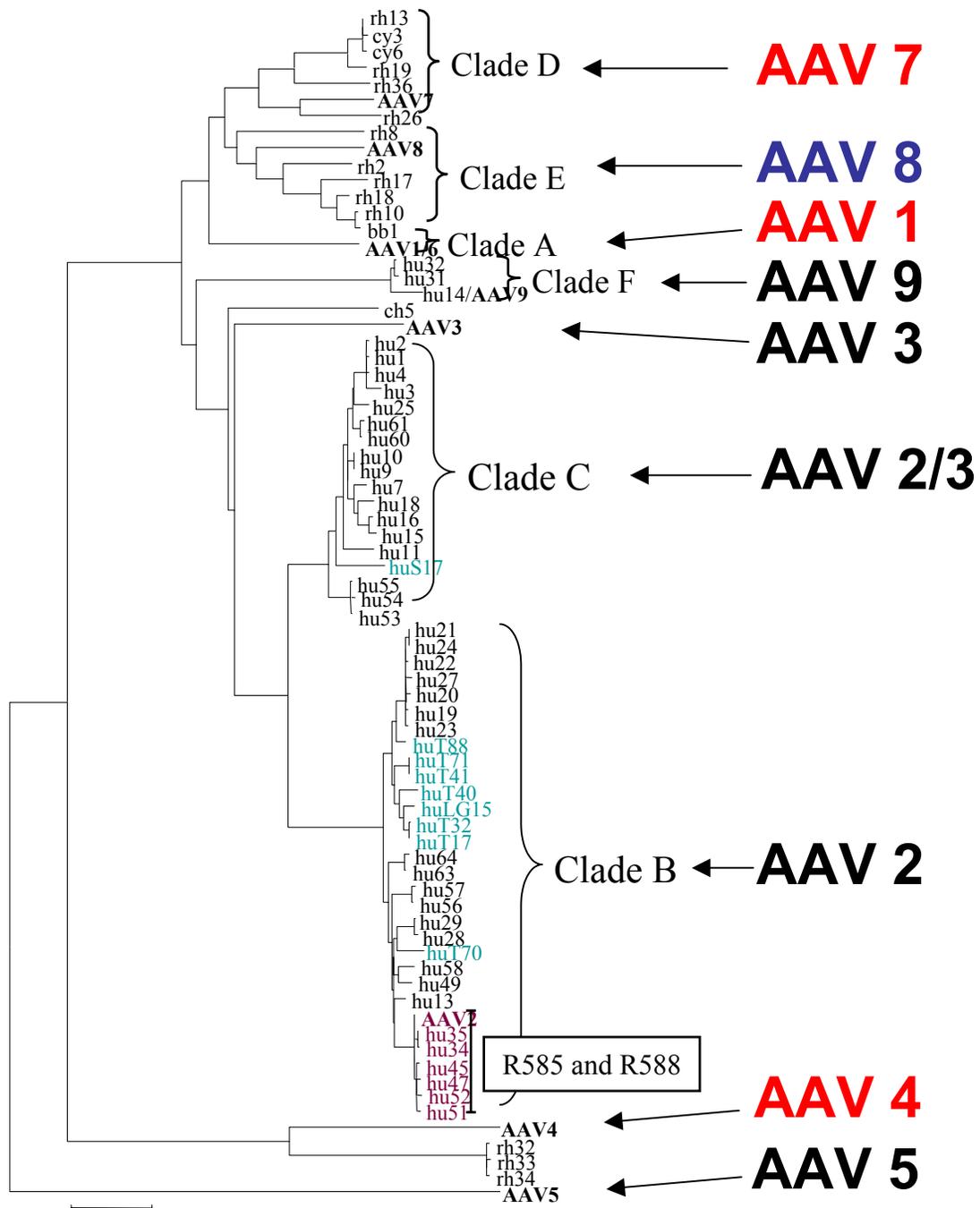
Clinical Trial

- **Initiated Phase I trial of tgAAC09 in humans Dec 2003**
- **Safety, immunogenicity endpoints**
- **Now fully enrolled (n = 50) in Belgium (St. Pierre and SGS) and Germany (Hamburg and Bonn)**

4. Emerging Concepts in AAV Biology

Wild-type AAV Infection of Primates

- **Historically 5 serotypes (1 - 5)**
- **More recently described**
- **Dissemination to many organs**
- **Multiple AAVs in a single organ**



Simian
 Human
 Both

AAV Neutralizing Antibodies

- AAV1 <10%
- AAV2 ~25%
- AAV3 ~25%
- AAV2/3 ?
- AAV4 <10%

- AAV5 <10%
- AAV7 <10%
- AAV8 <10%
- AAV9 ?

Fate of the Wild-type AAV Genome

- **Site-specific integration (*in vitro*)**
- **Host and viral elements required**
- ***In vivo* biology perhaps different**
- **Failure to detect site-specific integration**

Fate of the rAAV Genome

- Integration *in vitro* - like plasmid
- Viral genes missing
- *In vivo* - episomal concatamers (> 99.5%)
- Recent observation with plasmid

Partnerships

**Columbus Children's
Research Institute**

Reed Clark

Bruce Schnepf

Chun-Liang Chen

Mary Connell

Lauretta Turin

Ryan Jensen

David Montefiori (Duke)

Norm Letvin (Harvard)

**Targeted Genetics
Corporation**

**International AIDS
Vaccine Initiative**

**DAIDS/NIAID/NIH
(2-P01-AI26507)**

Partnerships

St. Pierre, Brussels

(N. Clumek)

SGS, Antwerpen

(E. Vets)

Universitätsklinikum Eppendorf, Hamburg

(J. van Luzen)

University of Bonn, Bonn

(J. Rockstroh)